

Neuregulin-1 is associated to postsynaptic sites contacting afferent C-type cholinergic terminals on lower motoneurons: changes in murine models of motoneuron diseases

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Background: Pioneering ultrastructural studies have defined different types of synapses (F-, S- and C-type boutons) on lower motoneurons (MNs). C-Type boutons are large nerve terminals characteristic of somatic α -MNs and display a subsynaptic cistern adjacent to postsynaptic membrane (1). The cholinergic nature of C-terminals has been established (2), and their origin from local interneurons has been deciphered (3). In a recent study (4) concerning neuregulin-1 (NRG-1) on phrenic MNs, it has been reported that this trophic factor is expressed in cholinergic terminals synapsing these MNs and a presynaptic localization of this protein was suggested.

Objectives: 1) To study the distribution of NRG-1 immunoreactivity (IR) amongst different MN groups; 2) to examine the localization of NRG-1 on spinal cord MNs by immunoelectron microscopy; 3) to perform a developmental analysis of NRG-1 expression; and 4) to explore NRG-1 changes in MNs of transgenic mouse models of spinal muscular atrophy (SMA) and ALS.

Methods: Immunofluorescence was performed on spinal cord from WT, SMN Δ 7 and SOD1 G93A mice and chick embryos. Analysis was done using confocal microscopy and ultrastructural immunolabeling.

Results: In normal adult WT animals, NRG-1-IR was concentrated close to VAcHT-positive cholinergic terminals around MN somata and proximal dendrites. However, a displacement of NRG-1 signal respect to presynaptic VAcHT-positive spots was evidenced, suggesting a different compartmentalisation. Ultrastructural examination revealed that NRG-1 was not associated with presynaptic terminals but with postsynaptic subsynaptic cisterns corresponding to C-terminals. A similar pattern of NRG-1-IR was found in brainstem MNs with the exception of those in oculomotor nuclei, in which NRG-1 expression was much lower. In chick embryo MNs, NRG-1 is early expressed (before E6); only at advanced developmental stages, NRG-1-IR appeared concentrated in relation to synaptic sites being downregulated after axotomy. In SMN Δ 7 and SOD1 G93A mice synaptic NRG-1 spots on MNs increased early during disease progression with a subsequent fall at the end stages.

Discussion and conclusion:

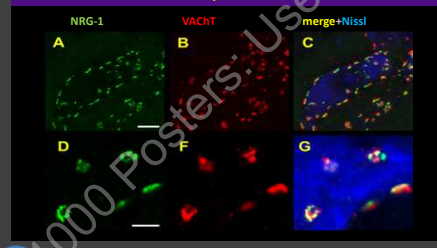
- 1) NRG-1 is concentrated at the subsynaptic cisterns of α -MN afferent cholinergic C-terminals.
- 2) ALS-resistant MNs at the oculomotor nuclei show low NRG-1 expression.
- 3) NRG-1 is developmentally regulated and depends on the maintenance of nerve muscle interactions.
- 4) The transient increase in NRG-1-positive spots on MN somata during SMA or ALS progression is in concordance with the described sprouting of C-terminals in ALS (4).

References:

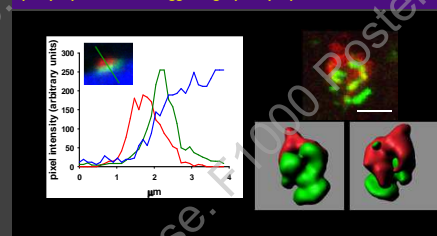
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2. Hellström J *et al.* *J Comp Neurol*. 1999; 411: 578-590.
3. Zagoraiou L *et al.* *Neuron* 2009; 64: 645-662.
4. Issa AN *et al.* *J Comp Neurol*. 2010; 518: 4213-4225.
5. Pullen AH, Athanasiou D. *Eur J Neurosci*. 2009; 29: 551-561.

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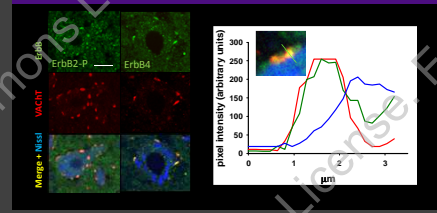
1 NRG-1 is concentrated close to cholinergic C-type afferent nerve terminals on spinal cord MN cell bodies



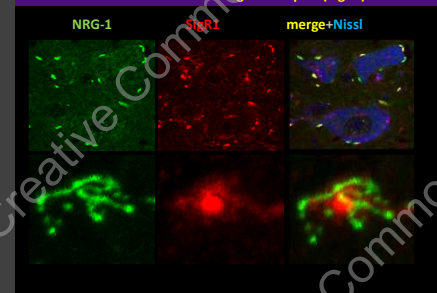
2 NRG-1 IR do not overlaps with cholinergic VAcHT-labelled presynaptic boutons suggesting a postsynaptic localization of NRG



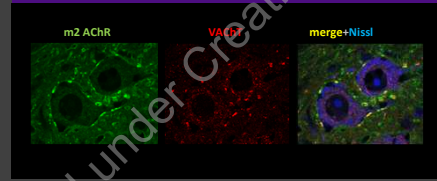
3 NRG-1 receptors ErbBs colocalize with VAcHT at C-type afferent boutons on MNs in face to adjacent to NRG-1 enriched postsynaptic sites at the MN surface



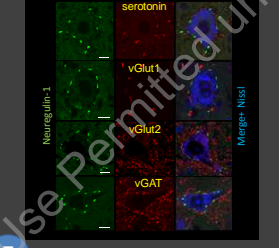
4 NRG-1 is accumulated in MN compartments narrowly linked with C-terminal-associated sub-surface cisternae enriched in Sigma receptor (SigR1)



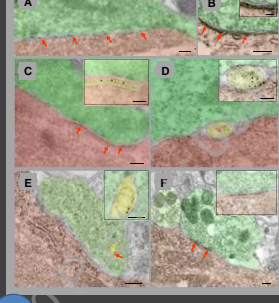
5 Muscarinic acetyl choline receptors at the MN surface are concentrated near VAcHT positive C-boutons



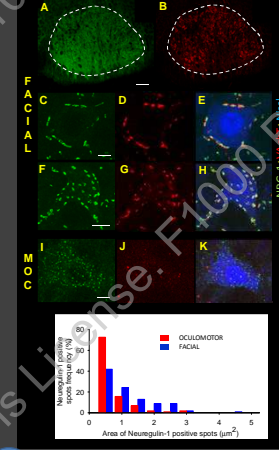
6 NRG-1 do not associate with MN afferent boutons other than cholinergic



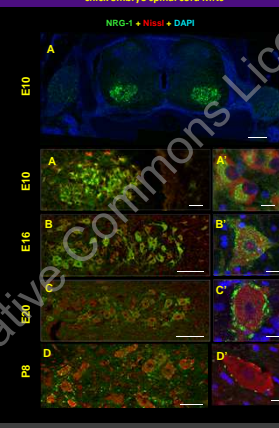
7 Ultrastructural immunolabeling demonstrates that NRG-1 is concentrated at the C-bouton-related sub-synaptic ER cistern at the MN soma



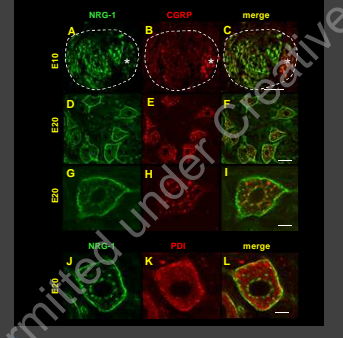
8 NRG-1 immunoreactivity in cranial MNs (facial and oculomotor)



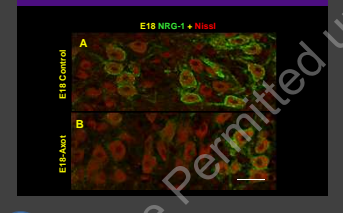
9 Development of NRG-1 immunoreactivity in the chick embryo spinal cord MNs



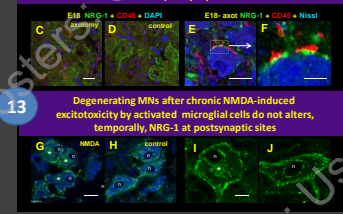
10 Compartmentation of NRG-1 within chick embryo spinal cord MNs: partial co-localization with the Golgi-secretory pathway marker GRP and ER marker PDI



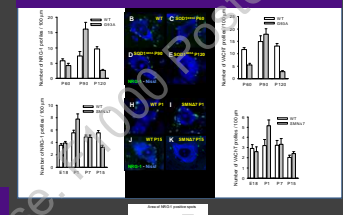
11 Downregulation of NRG-1 immunoreactivity in axotomized chick embryo MNs



12 Denervation of C-terminals by axotomy-induced synaptic stripping by activated microglial cells do not alters, temporally, NRG-1 at postsynaptic sites



13 Degenerating MNs after chronic NMDA-induced excitotoxicity by activated microglial cells do not alters, temporally, NRG-1 at postsynaptic sites



14 Changes in NRG-1 positive spots in MNs from mice models of ALS (SOD1G93A) and Spinal muscular atrophy (SMN Δ 7)



15 NRG-1 positive spots are present in human spinal cord MNs in control and ALS cases.

